Criterion™ Blotter

Instruction Manual

Catalog Numbers
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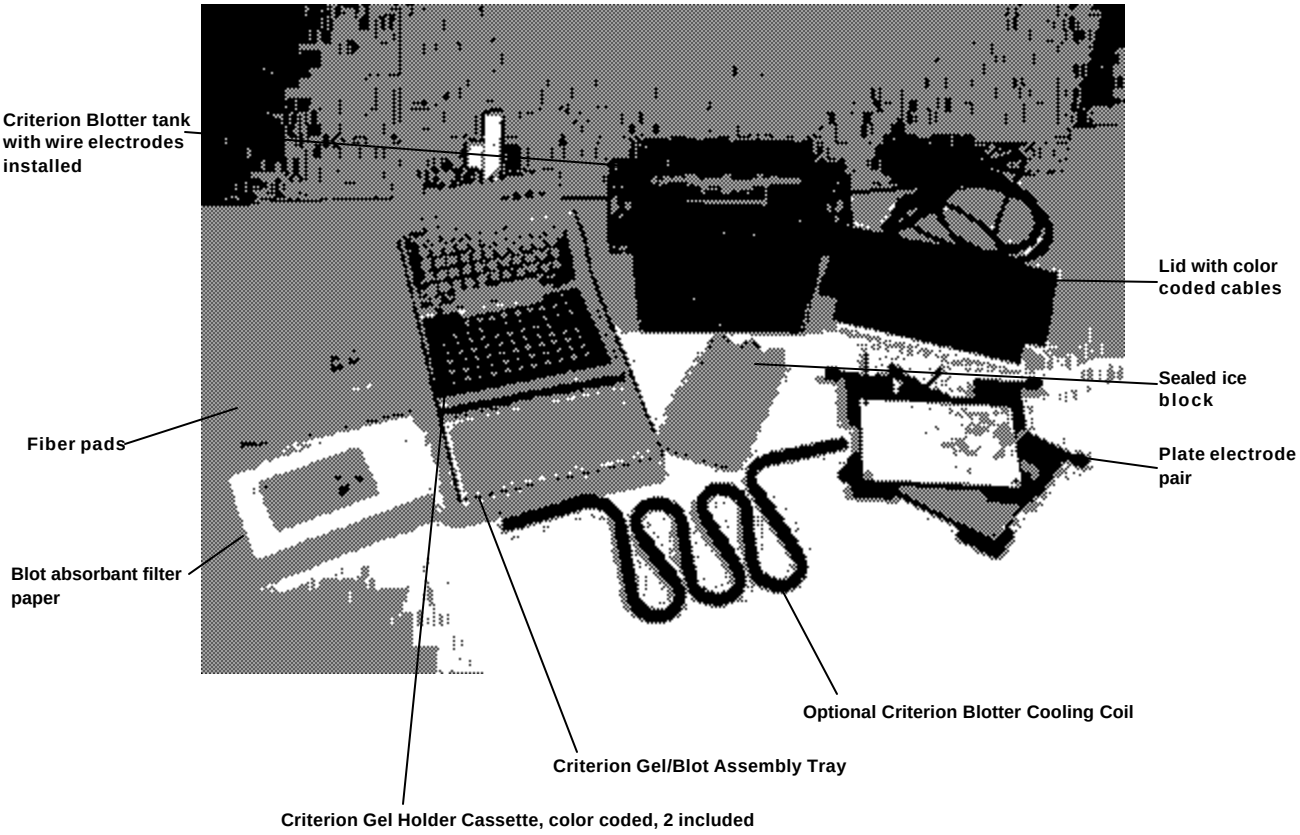
Section 1
General information

1.1 Introduction

The Criterion Blotter is an electrophoretic transfer cell designed for use with Criterion precast gels. The Criterion Blotter cell is available with plate or platinum wire electrodes. The Plate electrode pair consists of a platinum-coated titanium anode and a stainless steel cathode. Transfers are performed with either set of electrodes positioned 4.3 cm apart, using one or two gel holder cassettes positioned between the electrodes. This allows generation of a high intensity electrical field for an efficient transfer when used in combination with the PowerPac 200 Power Supply. Cooling is required for temperature control and can be achieved with the sealed ice block included with the cell, or with optional Criterion Cooling Coil. The Criterion Gel Blot Assembly Tray provides for lab tidy assembly of gel blot sandwiches and gel soaking, while minimizing the possibility of incorrect sandwich assembly. The roller is useful to ensure proper contact and removal of trapped bubbles during sandwich assembly.


Criterion Blotter tank with wire electrodes installed
Lid with color coded cables
Sealed ice block
Fiber pads
Plate electrode pair
Blot absorbant filter paper
Optional Criterion Blotter Cooling Coil
Criterion Gel/Blot Assembly Tray
Criterion Gel Holder Cassette, color coded, 2 included

1.2 Specifications

Criterion Blotter tank		
Overall dimensions		11.8 x 21.8 x 15 cm
Material		Molded polysulfone
Buffer requirement		1.3 liters
Electrodes		
Electrode Dimension		9.45 x 13.84 cm
Material	Support card	Red or black molded polysulfone
	Anode plate	Platinum coated titanium
	Cathode plate	Stainless steel
	Wire electrodes	Platinum wire
Distance Anode to Cathode		4.3 cm
Cassettes		
Cassette dimension		11.4 x 16.5 cm
Material		Red or black molded polysulfone
Maximum gel size		9.4 x 15 cm
Gel Capacity		2 Criterion or 4 Ready Gel precast gels
Gel/Blot Assembly tray		
Material		Molded polycarbonate
Overall dimensions		17.3 x 32.5 x 5.7 cm
Assembly compartment		14.3 x 17.3 x 3.2 cm
Soaking compartment		12.1 x 17.3 x 3.2 cm

1.3 Safety

Power to the Criterion Blotter cell is supplied by an external DC voltage power supply. This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground. All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

300 VDC	Maximum voltage limit
200 Watts	Maximum power limit
50 °C	Maximum ambient temperature limit

Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid, or when working with the cell in any way.

The Criterion Blotter is certified to meet EN61010-1* safety standard for safety of laboratory equipment. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to other equipment or accessories not EN61010-1 certified, even when connected to the Criterion Blotter.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer's warranty, void the EN61010-1 safety certification and create a potential safety hazard for the user.

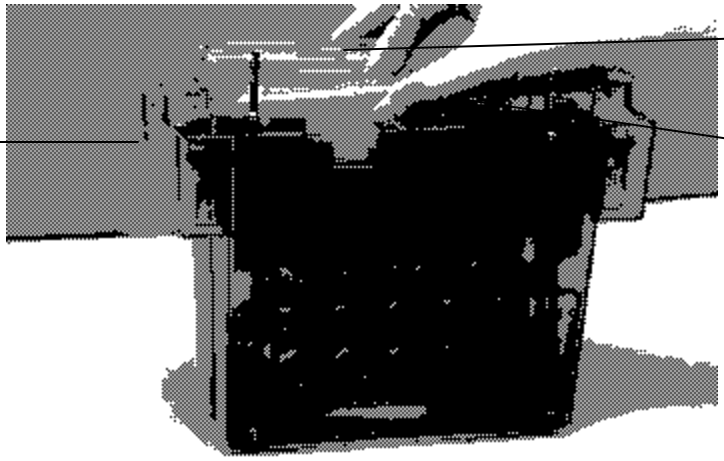
Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

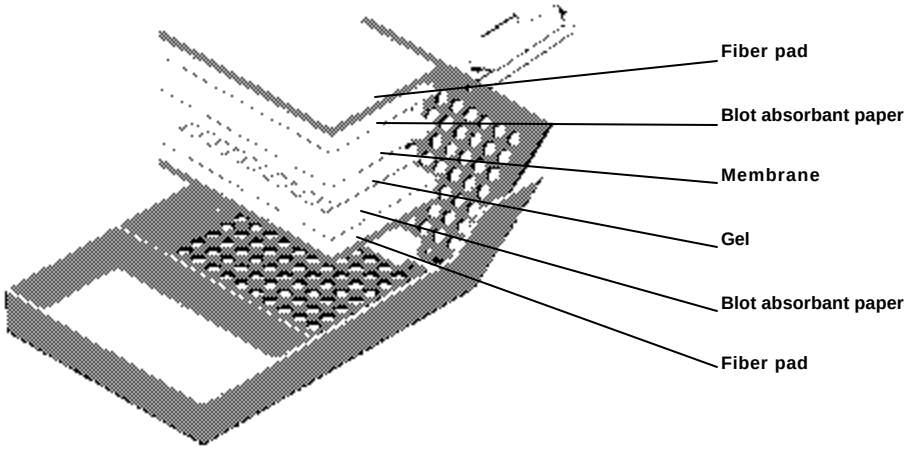
Section 2
Set Up and Basic Operation

Criterion Blotter Cell Assembly

2.1 Preparation for Blotting

1. Prepare the transfer buffer. (See Section 3.3 for buffer formulation. Using buffer chilled to 4 °C will improve heat dissipation.)
2. Equilibrate gel in transfer buffer for 15 minutes. Always wear gloves when handling membranes, filter paper, or gels to prevent contamination.
3. Set up transfer apparatus.
    a. Fill the Criterion Blotter tank with transfer buffer to about 50% of the fill volume.
    b. Place a magnetic stir bar inside the tank.
    c. Place the ice block in the ice block pocket in the back of the cell. Flip down the lever to hold the ice block down.
    d. Alternatively, the optional cooling coil can be used by connecting it to an appropriated recirculated water chiller and placing it in the grooves in the back of the tank.





4. Set up the gel/membrane sandwich:

   a. Pour some chilled transfer buffer into each compartment of the gel/blot assembly tray.

   b. Place the membrane (nitrocellulose, PVDF, etc.) in the front/small compartment of the tray. Let it soak while you set up Steps c–f.

   c. Place the cassette in the back/large compartment of the tray: Open the cassette so that the redside with handle is vertical (anode) and the black side (cathode) is laying horizontal and submerged in transfer buffer.

   d. Place a fiber pad on top of the black side cassette, submerged in buffer. Push on the fiber pad with gloved finger tips to thoroughly wet the pad.

   e. Place a piece of filter paper on top of the fiber pad (it will wet immediately).

   f. Gently place the pre-equilibrated gel on top of the filter paper. Use roller to remove any air bubbles that may be trapped underneath the gel.

   g. Take the membrane from the front compartment and place it on top of the gel taking care not to trap air. The membrane should not be moved or adjusted once it touches the gel because this can cause data ghost prints and artifacts. If you feel that you must adjust the membrane placement, use a fresh pre-wetted membrane. Use roller to roll out bubbles (see figure).

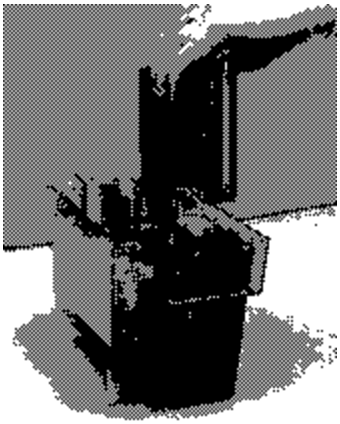
h. Place a piece of filter paper on top of the membrane. Run the roller gently over the top of the filter paper to remove any air bubbles trapped in the sandwich.

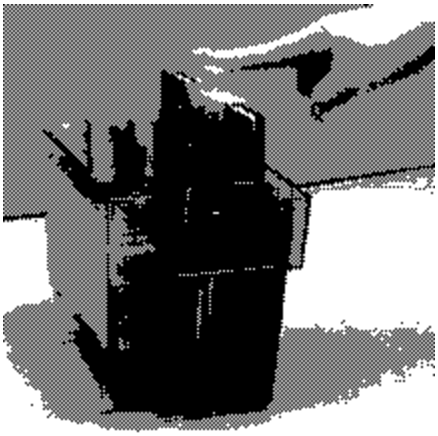
i. Wet a second fiber pad in the front compartment of the tray (where the membrane was soaking) again using finger tips to completely saturate the pad with transfer buffer. Then place the wet fiber pad on top of the second filter paper.

j. Lower the clamp-side of the cassette, and lock in the closed position (see figure).

2.2 Begin Transfer

a. Move the locked cassette into the groove in the blotter tank, aligning the red side of the card with the red electrode. Make sure the magnetic stirrer is free to move (see figure).





b. After both cassettes are in place, add the remaining transfer buffer to fill level marked on the tank.

c. Put on the lid, plug the cables into the power supply, and run the blot. Refer to Section 3 for run times with various buffers.

d. Transfer at a constant voltage using the Bio-Rad PowerPac 200 which has current capacity of 2A.

Upon completion of the run, disassemble the blotting sandwich and remove the membrane for development. Clean the cell, fiber pads, and cassettes with multiple rinses of deionized water.

2.3 Acidic Transfers

If transferring under acidic conditions, switch the gel and membrane in the set up instructions or simply reverse the orientation of the cassette when inserted in to the tank. Place the black side of the cassette next to the red electrode (anode). This will place the membrane on the cathode side of the gel. Under acidic conditions, proteins will transfer in the opposite direction going toward the negative cathode. **Do not reverse the electrodes themselves, or plug the banana plugs into the reverse poles. This will cause irreversible damage to the plate electrodes.**

Section 3
Transfer Conditions

3.1 General Guidelines to Transfer Buffers and Running Conditions

Tables 3.1 to 3.4 provide guidelines for power conditions using different buffers. Power conditions are provided for various run times. The transfer times will need to be increased for gradient gels, or they may be decreased if your protein of interest is low molecular weight and transfers quickly. The suggested conditions give at least 95% transfer of the sample proteins visible in the gel by Silver Stain Plus, catalog number 161-0449 (sensitive to ng level) and in the blot by Colloidal Gold, catalog number 170-6517 (sensitive to 4 ng).

Table 3.1 SDS-PAGE Gels

These conditions were determined empirically using 12.5% Tris-HCl Criterion gels and total proteins from *E.coli* lysates.

Buffer: 1X Tris/Glycine (see section 3.3 Buffer formulation)

	20% Methanol	**10% Methanol**	**15% Ethanol**
Criterion Blotter with plate electrodes	100 V 30 minutes	100 V 30 minutes	Not recommended*
Criterion Blotter with wire electrodes	100 V 60 minutes	100 V 30 minutes	Not recommended*

* Our tests show only 60% transfer of *E.Coli* proteins in 1 hour at 100V. The ethanol buffer might work if longer transfers are acceptable or if your target protein transfers under this condition.

Table 3.2 SDS PAGE Gels (CAPS based buffers)

These conditions were determined empirically using 12.5% Tris-HCl Criterion gels and total proteins from *E.coli* lysates.

Buffer: 10mM CAPS buffer (see Section 3.3 Buffer formulation)

	20% Methanol	**10% Methanol**	**15% Ethanol**
Criterion Blotter with plate electrodes	100 V 30 minutes**	100 V 30 minutes**	100 V 30 minutes
Criterion Blotter with wire electrodes	100 V 60 minutes**	100 V 30 minutes**	100 V 60 minutes

** We find nearly undetectable levels of proteins remaining in the gel, but some protein blow through is observed under this condition. PVDF is suggested for transfer of low molecular weight proteins.

Table 3.3 Native Gels

These conditions were determined empirically using 12.5% Tris-HCl Criterion gels and four native protein samples: cytochrome C (pI 9.6), lentil lectin (pI 8.2, 8.0, 7.8), carbonic anhydrous (pI 6.0) and glucose oxidase (pI 4.5).

Buffer: 1X Tris/Glycine (see Section 3.3 Buffer formulation)

The transfer of proteins from Native gels will depend on the size and pI of the protein relative to the pH of the buffer used during transfer. If the pI of the protein is greater than the pH of the transfer buffer, the protein carry a positive charge and will travel toward the negative electrode. The voltage suggested is a starting point. The transfer time will need to be determined empirically for your protein of interest.

Criterion Blotter with plate electrodes	Overnight (12 hrs) Max 10 V Max 50 mA	30 minutes 50 V 750–950 mA
Criterion Blotter with wire electrodes	Overnight (12 hrs) Max 10 V Max 50 mA	60 minutes 50 V 300–500 mA

Note: The power supply should be set on these maximum settings. The actual power supply reading may be lower throughout the run. These conditions are excellent for neutral proteins (pI~6.0), as we found at least 90% of the carbonic anydrous protein transferred successfully.

Table 3.4 DNA and RNA

These conditions were determined empirically using 5% uniform TBE Criterion gels and the low range Fluorescein labeled DNA standards (catalog number 170-3123).

Buffer: 1X TBE (see section 3.3 Buffer formulation)

Criterion Blotter with plate electrodes	Overnight (12 hrs) 10 V 100 mA	30 minutes 50 V 750–950 mA
Criterion Blotter with wire electrodes	Overnight (12 hrs) 20 V 100 mA	60 minutes 50 V 300–500 mA

Note: The power supply should be set on these maximum settings. The actual power supply reading may be lower throughout the run.

3.2 Notes on Electrophoretic Transfer Conditions

1. Pre-equilibration of gels

   All gels should be pre-equilibrated in transfer buffer prior to electrophoretic transfer (may not be necessary for native gels and nucleic acid gels where transfer buffer is generally the same as running buffer). Pre-equilibration will facilitate the removal of contaminating electrophoresis buffer salts and neutralization salts. If the salts are not removed, they will increase the conductivity of the transfer buffer and the amount of heat generated during the transfer. Also, gels will shrink to various degrees depending on the acrylamide percentage in methanol buffers. Equilibration allows the gel to adjust to its final size prior to electrophoretic transfer.

2. Current limits

   The PowerPac 200 Power Supply is capable of a 200 watt output. Unless a current limit is set, uncontrolled conductivity changes may result in full power being delivered to the Criterion Blotter cell. The gel holder and electrode cards may warp, and the transfer buffer may heat up (further increasing conductivity). This would result in a potential safety hazard. Refer to the PowerPac 200 Power Supply Instruction Manual for setting current limits and run times.

3. Polarity of transfer

   Do not reverse polarity with the plate electrodes. This will result in corrosion and rusting of the stainless steel cathode. If this should occur, the stainless steel should be cleaned with a mild abrasive cleanser to remove the rust.

4. Heat dissipation

   Two methods of heat dissipation are available. The efficient transfer and high intensity field of the Criterion blotter requires some method of heat dissipation. The sealed ice block cooling system is adequate for transfers of less than 1 hour where slightly elevated temperatures at the end of the transfer are acceptable. Where precise temperature control is required or transfers longer than 1 hour at high voltage conditions, the optional cooling coil connected to a refrigerated recirculating bath should be used. Placing the Criterion Blotter cell in the cold room is an inadequate means of controlling transfer buffer temperature. The tank of the Criterion Blotter cell is an effective thermal insulator, thus limiting the efficient dissipation of heat.

6. Use of a stir bar during transfer

   For all blotting applications a stir bar must be placed inside the Criterion Blotter cell so that the transfer buffer is stirred during the course of the experiment. This will help to maintain uniform conductivity and temperature distribution during electrophoretic transfer.

7. Transfer buffer pH

   Do not adjust the pH of transfer buffers unless specifically indicated. Adjustments of the pH of transfer buffers, when not indicated, will result in increased buffer conductivity. This is manifested by a higher than expected initial current output and a decreased resistance.

8. Transfer buffer recommendations

   Use only high quality, reagent grade methanol. Contaminated methanol can result in increased transfer buffer conductivity, as well as poor transfer of macromolecules. Reuse of transfer buffers is not advised, since these buffers have most likely lost their ability to maintain a stable solution pH during transfer. Dilution of transfer buffers below their recommended levels is also not advised, since this will decrease their buffering capacity.

9. Voltage limits

   Do not increase voltage settings beyond those indicated in Tables 3.1–3.4 for overnight operation. Buffer conductivity must be close to the current listed and a current limit should be set on the power supply. If overnight transfers at low voltages are ineffective for your application, and higher voltages are necessary, transfer times must also be adjusted to a shorter length. Failure to do so may result in a potential safety hazard.

10. These variables will change total resistance and thus the current readings:

    - Alterations in buffer make-up, *i.e.*, addition of SDS, or changes in ion concentration due to addition of acid or base to adjust the pH of the buffers.

- Gel pH, ionic strength, and percentage of acrylamide, especially if the gel has not been properly equilibrated.
- Number of gels; current increases slightly as the number of gels increases.
- Transfer temperature; current increases when temperature increases.
- Time in transfer at which reading was taken; current normally increases as the buffering capacity diminishes with progress of the run.

3.3 Buffer Formulation

All formulas provided below are for a total volume of 1 liter of buffer. 1.3 liters of buffer are required for the Criterion Blotter cell plus another 300 to 500 ml for assembly procedure for a total of 1.6 to 1.8 liters of buffer. It is efficient to use premixed buffer concentrates from Bio-Rad where available.

Do not add acid or base to adjust pH of the following buffers. Methanol should be analytical reagent grade, because metallic contaminants in low grade methanol will plate on the electrodes. Always add methanol/ethanol last.

Note: Some pH electrodes will not perform a proper measurement for the pH of Tris buffers. If the pH of the buffer is off, check to make sure the electrode is designed to work with Tris buffers. If the pH electrode functions properly for Tris buffers and the pH is below 8.0, remake the buffer.

1. SDS PAGE gels

Towbin Buffer with 20% Methanol

25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3

a) Use Premixed buffer concentrate solution:

100 ml of 10X Tris/Glycine buffer (catalog number 161-0734 1L bottles or catalog number 161-0757 5L cube)

700 ml of dd H_2O

200 ml of methanol

b) From dry reagents:

Mix 3.03 g Tris, 14.4 g glycine in 600 ml of dd H_2O, add distilled deionized water (dd H_2O) to 800 ml, add 200 ml Methanol.

Towbin Buffer with 10 % Methanol

25 mM Tris, 192 mM glycine, 10% v/v methanol, pH 8.3

a) Use Premixed buffer concentrate solution:

100 ml of 10X Tris/Glycine buffer (catalog number 161-0734 1L bottles or catalog number 161-0757 5L cube)

800 ml of dd H_2O

100 ml of methanol

b) From dry reagents:

Mix 3.03 g Tris, 14.4 g glycine in 600 ml of dd H_2O, add distilled deionized water (dd H_2O) to 900 ml, add 100 ml Methanol.

CAPS Buffer with 20 % Methanol

10 mM CAPS (3-(cyclohexylamino)-1-propane sulfonic acid), 20% v/v methanol, pH 11

From dry reagents:

Mix 2.21g CAPS in 600 ml of dd H_2O, ADJUST the pH to 11.0 with NaOH, add distilled deionized water (dd H_2O) to 800 ml, add 200 ml Methanol.

CAPS Buffer with 10 % Methanol

10 mM CAPS (3-(cyclohexylamino)-1-propane sulfonic acid), 10% v/v methanol, pH 11

From dry reagents:

Mix 2.21g CAPS in 600 ml of dd H_2O, ADJUST the pH to 11.0 with NaOH, add distilled deionized water (dd H_2O) to 900 ml, add 100 ml Methanol.

CAPS Buffer with 15% Ethanol

10 mM CAPS (3-(cyclohexylamino)-1-propane sulfonic acid), 15% v/v ethanol, pH 11

From dry reagents:

Mix 2.21g CAPS in 600 ml of dd H_2O, ADJUST the pH to 11.0 with NaOH, add distilled deionized water (dd H_2O) to 850 ml, add 150 ml Ethanol.

2. Native gels

25 mM Tris, 192 mM glycine, pH 8.3

a) Use Premixed buffer concentrate solution:

100 ml of 10X Tris /Glycine buffer (catalog number 161-0734 1L bottles or catalog number 161-0757 5L cube)

900 ml of dd H_2O

b) From dry reagents:

Add 3.03 g Tris, 14.4 g glycine to distilled deionized water (dd H_2O) then mix and add dd H_2O to a final volume of 1 liter.

3. Nucleic Acid gels

TBE (Tris-Borate EDTA)

89 mM Tris borate, 2 mM EDTA pH 8.3

100 ml of 10X TBE buffer (catalog number 161-0733 1L bottles or catalog number 161-0770 5L cube)

900 ml of dd H_2O

TAE (Tris-Acetate EDTA)

40 mM Tris-Acetate 1 mM EDTA

20 ml of 50X TAE buffer (catalog number 161-0743 1L bottles or catalog number 161-0773 5L cube)

980 ml of dd H_2O

Section 4
Strategies for Optimizing Electro-Elution

4.1 Optimizing Protein Transfer

Generally, quantitative elution of denatured high molecular weight proteins is difficult. The following tactics, alone or in combination, will increase transfer efficiency.

1. Failure of molecules to bind efficiently to the membrane, caused by poor gel-membrane contact, is often confused with inefficient elution. Poor contact is usually due to excess moisture in the gel-membrane interface. Proper technique and the use of a test tube or roller should assure good contact. Proper selection of filter paper spacers will help assure good compression. Gel and membrane equilibration in transfer buffer for at least 15 minutes prior to transfer will help prevent shrinking of either component during transfer, and will eliminate reactants such as urea or SDS from the gel.

2. Increase transfer time. An initial control should be performed to determine the time required for complete transfer.[17,24] Times may vary from as little as 30 minutes to as long as overnight. Remember all overnight applications should be performed at 30–50 volts to minimize heating problems. (For long transfers at elevated voltages use the criterion cooling coil option.)

3. Increase the power. Initial controls should be performed to evaluate the efficiency of increasing the V/cm as well as its effects on the temperature of transfer. The temperature increase may change buffer resistance and subsequent power delivered, as well as the state of protein denaturation, thus affecting transfer efficiency.

4. Vary buffer type and pH

    a. Reduce buffer strength. Dilution of transfer buffer results in lower current at any given voltage. This will allow the use of higher voltages without excessive heating.

    b. Maximize charge-to-mass ratio. It appears that alcohols present in SDS transfer buffer strip SDS from proteins. Basic proteins in Tris, glycine, methanol buffer at pH 8.3 may assume a state near isoelectric neutrality and thus transfer poorly. For example, lysozyme exhibits this behavior. Buffers with pH of 9.5 to 10.0 have shown much better elution and binding characteristics for basic proteins such as lysozyme and histones.[41]

    c. Different buffer types at similar V/cm may yield different efficiencies. Generally Tris buffers allow more efficient transfer than acetate or phosphate buffers.

    d. Addition of 0.1% SDS detergent to Tris, glycine, methanol buffer has been reported to increase transfer efficiency.[24] SDS, however, increases relative current, power, and heating. Also, temperatures below 10 °C may precipitate the SDS so the starting buffer temperature will be higher. SDS may also affect the antigenicity of some proteins. SDS will aid in eluting the proteins from the gel, but it may reduce the binding efficiency of those proteins to the nitrocellulose membrane.[4 2]

    e. Eliminate alcohol from the transfer buffer. Alcohol in the transfer buffer improves binding of SDS proteins to nitrocellulose. Elimination of alcohol results in increased transfer efficiency but diminishes binding to nitrocellulose. Transfer efficiency is increased because alcohol causes gel pores to contract resulting in fixation of large molecular weight proteins within the gel matrix. Use of PVDF membrane for SDS protein transfers may reduce the alcohol requirement, and constitutes a logical strategy for analysis of high molecular weight or difficult-to-transfer proteins.[26, 27]

5. Alter membrane type. As mentioned in 5e, PVDF membrane allows transfer in reduced alcohol(see Section 5.1). PVDF can increase the binding of low molecular weight proteins that sometimes blow through nitrocellulose when transfers are long enough or intense enough to transfer high molecular weight proteins. Use Immun-Blot PVDF if the blot will be developed with immunochemicals. Use Sequi-Blot PVDF for proteins that will be sequenced or delivered to mass spec.

4.2 Optimizing DNA and RNA Transfer

Problems with elution of nucleic acids can be solved by altering the gel percentage. It may be somewhat more difficult to quantitatively transfer large amounts of DNA used in genomic blots. The following tactics should be considered for optimizing elution in such transfers.

1. Alter gel composition.
   a. Lower % total monomer or % crosslinker for polyacrylamide gels.
   b. Lower % agarose. This allows better elution of high molecular weight DNA.
2. Alter DNA denaturants. It has been found that glyoxal denaturation allows more efficient elution of DNA than NaOH. Boiling polyacrylamide gels to denature DNA has also been found to give excellent results.[11] Base denaturation often causes polyacrylamide gels to weaken and stick to blotting membranes.

Section 5
Choice of Blotting Membranes

5.1 Protein Blotting Membranes

PVDF Membrane

Bio-Rad offers PVDF (Polyvinylidene difluoride) membranes ideal for immunoassays of blotted proteins (Immun-Blot PVDF) or amino-terminal sequencing and amino acid analysis (Sequi-Blot PVDF). PVDF retains proteins under extreme conditions of exposure to acidic or basic conditions, and in the presence of organic solvents. Greater protein binding capacity allows for better retention of easily transferred proteins, while allowing more time or higher voltages to transfer difficult or larger proteins. Greater retention during sequencing manipulations enhances the likelihood of obtaining information from rare, low abundance proteins, by increased initial coupling and higher repetitive yields. In addition, PVDF membrane exhibits better binding efficiency of blotted material in the presence of SDS in the transfer buffer. **PVDF must first be wetted in 100% MeOH**

Nitrocellulose Membrane

Nitrocellulose membranes have been used extensively for protein binding and detection.[7,20,23,24,27] They can be easily stained for total protein by a dye stain (Amido Black, Coomassie® Blue, Ponceau S, Fast Green FCF, etc.),[2 7] or the more sensitive Colloidal Gold Total Protein Stain, and also allow either RIA, FIA or EIA. Nonspecific protein binding sites are easily and rapidly blocked, avoiding subsequent background problems. No pre-activation is required. Low molecular weight proteins (especially <20,000 daltons) may be lost during post transfer washes, thus limiting detection sensitivity.[1 9] Smaller pore size nitrocellulose membrane (0.2 µm), has been shown to be effective in eliminating this loss. Large proteins (= 100,000 daltons) denatured by SDS may transfer poorly due to the addition of alcohol to the transfer buffer. Alcohol increased binding of SDS proteins to nitrocellulose, but decreased

pore sizes in the gel. Elimination of alcohol from SDS-protein transfers results in considerably diminished binding. Adding SDS (up to 0.1%) to the transfer buffer increases the transfer efficiency of proteins, but reduces the amount of binding to the membrane. Also, SDS increases the conductivity of the buffer and the heat generated during transfer.

5.2 DNA and RNA Blotting Membrane

Zeta-Probe® Nylon Membrane

Nitrocellulose is not a suitable medium for electrophoretic transfer of nucleic acids, as high concentrations of salt (= 10 x SSC) are required for efficient binding.[13] Molecules = 500 bp are not bound at all, even at high salt. Low resistance results when an electric current is passed through a solution of high salt. This causes potentially damaging high currents (and power) at very low voltages. Since V/cm is the eluting force, inefficient transfer occurs under conditions required for proper binding. Zeta-Probe membrane allows efficient binding of all sizes of single stranded DNA and RNA in the presence of low ionic strength buffers.[1 3] Zeta-Probe membrane is an ideal alternative to nitrocellulose for the analysis of nucleic acids. Binding is more stable through post transfer washes, and reprobing may be performed as many as 10 times.

Table 5.1 Guide to Protein Blotting Membranes

A variety of blotting membranes is available for immunoblotting, each with particular advantages depending on the needs of the experiment. The physical properties and performance characteristics of a membrane should be evaluated in selecting the appropriate transfer conditions.

Membrane	Binding Capacity Pore Size	(µg/cm2)	Notes
Nitrocellulose	0.45 µm 0.2 µm	80–100	General purpose protein blotting membrane
Supported Nitrocellulose	0.45 µm 0.2 µm	80–100	Pure nitrocellulose cast on an inert synthetic support; Nitrocellulose 0.2 µm increased strength for easier handling and for reprobing.
Immun-Blot PVDF	0.2 µm	150–160	High mechanical strength and chemical stability, used for immune detection western blotting; low background to signal ration, enhanced binding in the presence of SDS. Must be wet in alcohol before equilibration in buffer.
Sequi-Blot PVDF	0.2 µm	170–200	High mechanical strength and chemical stability, used for protein sequencing, enhanced binding in the presence of SDS. Must be wet in alcohol before equilibration in buffer.

Section 6
Troubleshooting Guide

6.1 Electrophoretic Transfer

Poor or no electrophoretic transfer (as detected by staining the gel)

1. Transfer apparatus is assembled incorrectly, and the proteins are moving in the wrong direction.
    - The gel/membrane sandwich may be assembled in the wrong order or the cassette is inserted in the tank facing the opposite orientation. Check the polarity of the connections to the power supply.
2. Detection system is not working or not sensitive enough.
    - Include proper positive and negative control antigen lanes to test for detection kit sensitivity. Consult kit manual.
3. Transfer time is too short.
    - Increase the transfer time.
4. Charge-to-mass ratio is incorrect (Native transfers).
    - Try a more basic or acidic transfer buffer to increase protein mobility. Proteins near their isoelectric point at the pH of the buffer will transfer poorly. (It has been suggested that buffer pH should be 2 pH units higher or lower than the pI of the protein of interest for optimal transfer efficiency.)

5). Power supply circuit is inoperative, or an inappropriate power supply was used.
    - Check the fuse. Be sure the voltage and current output of the power supply match the needs of the blotting instrument.
6. Methanol in the transfer buffer is restricting elution.
    - Reduction of methanol results in increased transfer efficiency of proteins from the gel, but it also diminishes binding to nitrocellulose and PVDF.

Protein is precipitating in the gel

1. Try using SDS in the transfer buffer. SDS can increase transfer efficiency, but can also reduce binding efficiency to nitrocellulose and affect reactivity of some proteins with antibodies.

Swirls or missing bands; diffuse transfers

1. Poor contact between the membrane and the gel. Air bubbles or excess buffer remain between the blot and gel.
    - Use the included roller, test tube, or pipet as a rolling pin, and roll over the membrane carefully in both directions until air bubbles or excess buffer is removed from between gel and membrane, and complete contact is established.
    - Use thicker filter paper in the gel/membrane sandwich.
    - Replace the fiber pads. Pads will compress with time, and will not hold the membrane to the gel.

2. The membrane is not properly wet or has dried out.
    - White spots on the nitrocellulose membrane indicate dry areas where protein will not bind. If wetting does not occur immediately by immersion of the sheet in transfer buffer, heat distilled water until just under the boiling point, and soak the membrane until completely wet. Equilibrate in transfer buffer until ready for use.
    - Because of the hydrophobic nature of PVDF, the membrane must be prewet in methanol prior to equilibration in aqueous transfer buffer. Follow the directions in the product insert.
3. The gel electrophoresis may be at fault.
    - Artifacts of electrophoresis may be produced by poor polymerization, inappropriate running conditions, contaminated buffers, sample overload, etc. Consult your electrophoresis manual for more details.

Gel cassette pattern transferred to blot

1. Contaminated or thin fiber pads are used.
    - Replace the fiber pads, or thoroughly clean the contaminated pads.
2. The transfer buffer is contaminated.
    - Make fresh solutions.

Poor Binding to the Membrane—Nitrocellulose

1. 20% methanol in the transfer buffer is optimal for protein binding.
    - Make sure the buffer contains the proper amount of methanol.
2. Proteins may be transferring through the nitrocellulose.
    - Use PVDF or nylon (higher binding capacities) or 0.2 µm nitrocellulose (smaller pore size). Decrease the voltage.
3. Proteins <15,000 daltons may show diminished binding to 0.45 µm nitrocellulose, or may be washed from the membrane during assays.
    - Use PVDF or nylon membrane, which have higher binding capacities.
    - Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more stringent washing conditions.
4. SDS in the transfer buffer will reduce binding efficiency of proteins.
    - Reduce or eliminate the SDS from the transfer buffer.
5. The membrane may not be completely wet.
    - White spots on the membrane indicate dry areas where protein will not bind. If wetting does not occur immediately by immersion of the sheet in transfer buffer, heat distilled water until just under the boiling point, and soak the membrane until completely wet. Equilibrate in transfer buffer until ready for use.

Poor Binding to the Membrane—PVDF

1. The membrane may not be completely wet.
    - Because of the hydrophobic nature of PVDF, the membrane must be prewet in alcohol prior to equilibration in aqueous transfer buffer. Follow the directions in the product insert.

2. The membrane may have been allowed to dry during handling.

    - A completely wet membrane has a gray, translucent appearance. White spots will form on the surface of the membrane, indicating that it has been allowed to dry. Since proteins will not bind to the dry spots, rewet the membrane with methanol and re-equilibrate in transfer buffer.

Power is too low/high

- Always check the current at the beginning of the run. The current may be too low for a particular voltage setting. If the buffer is prepared improperly, the conductivity may be too low, and not enough power will be delivered to the cell. See the power guidelines for specific applications in Section 3.
- Remake the buffer or alter the voltage (increase or decrease).
- Try changing the intensity of blotting (wire vs. plate electrodes).

Immune-Specific Detection

Overall High Background, low signal, or lack of development of positive control.

- Consult instructions for immune detection kit or reagents.

Total Protein Detection

Consult user manual for stain or detection kit.

Section 7
Maintenance

Cleaning: Use mild soap and warm water to clean the electrodes, cassettes, and buffer tank. Use special care when cleaning the electrode cards or plate electrodes. Avoid stretching or breaking the platinum wires. Avoid scratching or marring the platinum plate. Do not use abrasives or strong detergents. The cathode plate (stainless steel) can be cleaned with a mild abrasive to remove salt that may be deposited during normal operation. Rinse the fiber pads under hot water and then in distilled deionized water.

Chemical compatibility: The Criterion Blotter cell components are not compatible with chlorinated hydrocarbons (*e.g.*, chloroform), aromatic hydrocarbons (*e.g.*, toluene, benzene), or acetone. Use of organic solvents voids all warranties.

Section 8
Product Information

Catalog Number	Product Description
170-4070	**Criterion Blotter–Plate electrodes**, includes, Cell assembled with plate electrodes, lid with cables, 2 Criterion gel holder cassettes, filter paper pack, fiber pad pack, gel blot assembly tray, sealed ice cooling unit, manual, roller
170-4071	**Criterion Blotter–Wire electrodes**, includes, Cell assembled with wire electrodes, lid with cables, 2 Criterion gel holder cassettes, filter paper pack, fiber pad pack, gel blot assembly tray, sealed ice cooling unit, manual, roller

Catalog Number	Product Description
170-4072	**Criterion Blotter–170-4070 (with Plate electrodes) and PowerPac 200 Power Supply**, 110/120 V
170-4073	**Criterion Blotter–170-4070 (with plate electrodes) and PowerPac 200 Power Supply**, 220/240 V
170-4074	**Criterion Blotter–170-4071 (with wire electrodes) and PowerPac 200 Power Supply**, 110/120 V
170-4075	**Criterion Blotter–170-4071 (with wire electrodes) and PowerPac 200 Power Supply**, 220/240 V
170-4076	**Optional Criterion Blotter Cooling Coil**
165-5052	**PowerPac 200 Power Supply**, 110/120 V
165-5053	**PowerPac 200 Power Supply**, 220/240 V
Criterion Blotter Cell Accessories	
170-4080	**Criterion Gel Holder Cassettes**, 1
170-4081	**Criterion Blotter Platinum Anode Plate Electrode**
170-4082	**Criterion Blotter Stainless Steel Cathode Plate Electrode**
170-4083	**Criterion Blotter Standard Wire Electrode Card**, anode
170-4084	**Criterion Blotter Standard Wire Electrode Card**, cathode
170-4085	**Filter Paper, 9.5 x 15.2 cm**, 50
170-4086	**Fiber Pads, 9.5 x 15.2 cm**, 4
170-4087	**Sealed Ice Cooling Unit**, 2
170-4089	**Criterion Gel/Blot Assembly Tray**
165-1284	**Roller**

Section 9
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Section 10
Warranty

The Criterion Blotter electrophoretic transfer cell is warranted for one (1) year against defects in materials and workmanship. If any defects occur during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts without charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by deliberate or accidental misuse.
5. Corrosion due to use of improper solvent or sample.

Use with chlorinated hydrocarbons (*e.g.*, chloroform), aromatic hydrocarbons (*e.g.*, toluene, benzene), or acetone.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

Warranty Information

Model _____

Catalog Number _____

Date of Delivery _____

Serial Number _____

Invoice Number _____

Purchase Order No _____

Bio-Rad Laboratories

Life Science Group

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